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PREDISPOSITION OF TOMATO TO BACTERIAL CANKER[1]

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INTRODUCTION

In a recent paper, Foster and Walker (*3*)[3] reported the results of a study of wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen) in tomato plants grown before inoculation under a number of different environmental conditions. It was shown that while temperature, moisture, and light had an influence on the rate and degree of disease development after infection, they also had a distinct and sometimes a different influence on the disease through their effect upon the host previous to infection. Since effects of predisposition have received little attention in most environal studies this investigation was extended to include bacterial canker (*Corynebacterium michiganense* (Sm.) H. L. Jensen), another vascular disease in which the pathogen is confined in the early stages of disease development to the phloem.

METHODS AND MATERIALS

Plants of the Bonny Best variety of tomato (*Lycopersicon esculentum* Mill.) were grown in clean, white quartz sand, watered with distilled water. When the first leaf began to expand, usually at 2 to 3 weeks, the plants were set in a mixture of 3 parts of compost soil and 1 part of sand. They were then exposed to the various predisposition treatments for a period of 5 to 6 weeks.

Plants treated at controlled air temperatures were placed in greenhouses in which the temperature was regulated by automatic control. When controlled soil temperatures were concerned in the treatment, plants were grown in Wisconsin soil temperature tanks. The growth of uninoculated control plants in a given treatment was measured by taking the fresh weight of plants, including the roots.

Because the canker organism tended to lose virulence when grown on artificial media, it was maintained in tomato plants. To facilitate storing the organism in tomato material over long periods of time, infected tomato stems and petioles were sectioned into 2-inch pieces, placed in tin containers, frozen immediately and kept frozen until needed. When test inoculations were made from material kept in this manner 1 week, 1 month, 2 months, 3 months, and 5 months after freezing, growth in culture was typical and virulence was not lessened, as shown by inoculations. Typical wilting symptoms of bacterial

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[3] Italic numbers in parentheses refer to Literature Cited, p. 186.

canker developed in 7 to 14 days after inoculation. Before each inoculation a single colony isolate, selected from a dilution plate culture made from the frozen material, was used to start mass cultures.

Inoculum was made in nutrient-dextrose broth [4] which was prepared in 3-liter lots in 6-liter Florence flasks. The medium was adjusted to pH 6.5. Each flask was inoculated with a 5-ml. bacterial suspension in sterile, distilled water made from a 7-day-old slant culture derived from a single colony as indicated above. Inoculated flasks were then placed at 24° C. on a motor-driven shaker. At the end of 5 days the cultures were centrifuged in a Sharples supercentrifuge at 40,000 r. p. m. and 200 ml. for 1 minute. This separated the bacteria from the solution and eliminated any growth products or toxins which might have accumulated in the medium. Approximately 15 gm. of bacterial concentrate was obtained from 3 liters of culture medium. The concentrate was placed in a sterile petri dish at 4° C. overnight, and was then suspended in saline (0.85 percent sodium chloride) at a rate of 7 gm. per liter. This suspension was used as the inoculum.

Plants were dug and after the roots were washed in tap water they were inoculated by clipping the taproot with scissors, beneath the surface of the suspension, about a centimeter from the tip. The control plants were treated in a similar manner, except that saline alone was used instead of the bacterial suspension. The plants were replanted in the pots from which they had been removed, except as noted.

Disease readings were taken periodically after the onset of symptoms and each plants was placed in one of a series of disease classes set up as follows:

0—No symptom expression.
20—Wilting of one leaf, usually a unilateral wilting of leaflets on one leaf.
40—Wilting of more than one leaf.
60—Wilting of leaves and the growing tip.
80—Wilting of all leaves and the growing tip.
100—Plants dead.

A disease index for each treatment replicate was secured by multiplying the number of plants in each class by the class number, adding the products and dividing the sum by the total number of plants in that treatment replicate.

EXPERIMENTAL RESULTS

RELATION OF TEMPERATURE TO BACTERIAL GROWTH

The influence of temperature on bacterial growth was studied in nutrient-dextrose broth tubes containing 8 ml. of the medium. Each tube was inoculated with a loop of bacterial suspension. Twenty-one inoculated tubes and 7 blanks were placed at each of the following temperatures: 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. On alternate days growth was measured by relative turbidity taken with a Klett-Summerson photoelectric colorimeter using filter No. 420. Three inoculated tubes from each temperature were read. As shown in table 1, the optimum temperature for growth in nutrient-dextrose broth was 32°. Growth decreased with temperature, becoming very slow at 8° and no measurable amount appearing at 4° until the eleventh day. No growth was observed at 36°. In a second experiment the

[4] 10 gm. dextrose; 10 gm. Bacto-peptone; 3 gm. beef extract; 1,000 ml. distilled water.

growth curve was similar. The optimum determined here is slightly higher than that reported for the same species by Bryan (*2*) and by Blood (*1*).

TABLE 1.—*Effect of temperature on growth of the canker organism in nutrient-dextrose broth*

Temperature	Average turbidity at indicated number of days after inoculation				
	2	4	6	8	11
°C.					
4	0	0	0	0	2.5
8	0	0	0	0	19.5
12	0	0	18.0	21.5	33.0
16	0	34.3	34.5	33.0	39.0
20	16.5	21.4	37.5	37.0	54.5
24	17.0	18.0	43.0	46.5	60.7
28	6.5	41.5	50.5	60.5	76.7
32	16.4	26.0	52.5	68.0	91.5
36	0	0	0	0	0

RELATION OF AIR AND SOIL TEMPERATURE TO THE DISEASE

Blood (*1*) studied the effects of soil and air temperature on development of bacterial canker. He inoculated uniform plants and grew them at various constant soil temperatures ranging from approximately 16° to 36° C. at each of the following air temperatures: 16°, 20°, 24°, and 28°. The soil was maintained at a moisture level favorable for host growth. From four experiments he concluded that a soil temperature of about 28° was optimum for disease development at each of the four air temperatures. When uniform plants were inoculated and placed at air temperature 16°, 20°, 24°, 28°, 32°, and 36° C., the optimum air temperature for disease development was 28°, while 24° and 32° were almost as favorable. Growth of the uninoculated control plants was found to be influenced by air and by soil temperature. Plants grown at high air and low soil temperature grew better than plants at low air and high soil temperature. Maximum growth occurred where both soil and air were near 28° C. Thus the disease progressed most rapidly at the combination of air and soil temperature most favorable to the development of the host.

In the present investigation, the effect of various combinations of air and soil temperature upon disease development was studied further. In each treatment of the first experiment the same soil and air temperature was used for both the pre- and the post-inoculation period. Twelve crocks, 5 plants per crock, were used at each soil temperature. The soil and air temperature combinations are shown in table 2. Soil moisture was maintained at a level favorable to host growth. At 38 days after transplanting, when the plants had become well established and when they showed the effects of growing under the different conditions, plants in 10 crocks per soil temperature were inoculated while plants in 2 crocks were treated in saline to serve as controls. Growth as measured by the fresh weights of uninoculated plants is shown in table 2. Poor growth at all soil temperatures in 28° air was due, in large part, to the low light intensity in the 28° house. Growth of plants throughout the experiment was best in soil at 24° or 28° when the air was at 16°, 20°, or 24°. Maximum growth was in 24° soil and 24° air.

TABLE 2.—*Effect of soil and air temperature on disease development and on growth of uninoculated plants*

Temperature		Average weight uninoculated plants		Disease index at 37 days
Air	Soil	At time of inoculation	At end of experiment	
° *C.*	° *C.*	*Grams*	*Grams*	
16	16	1.3	3.3	46.6
	28	2.4	35.2	87.6
20	16	1.6	7.4	36.2
	24	3.6	35.0	80.1
	32	2.9	30.9	82.9
24	16	2.4	14.3	28.7
	24	5.4	36.1	78.6
	32	4.5	25.7	82.1
28	16	1.2	4.2	58.9
	24	2.9	7.7	71.9
	32	2.1	8.0	52.8

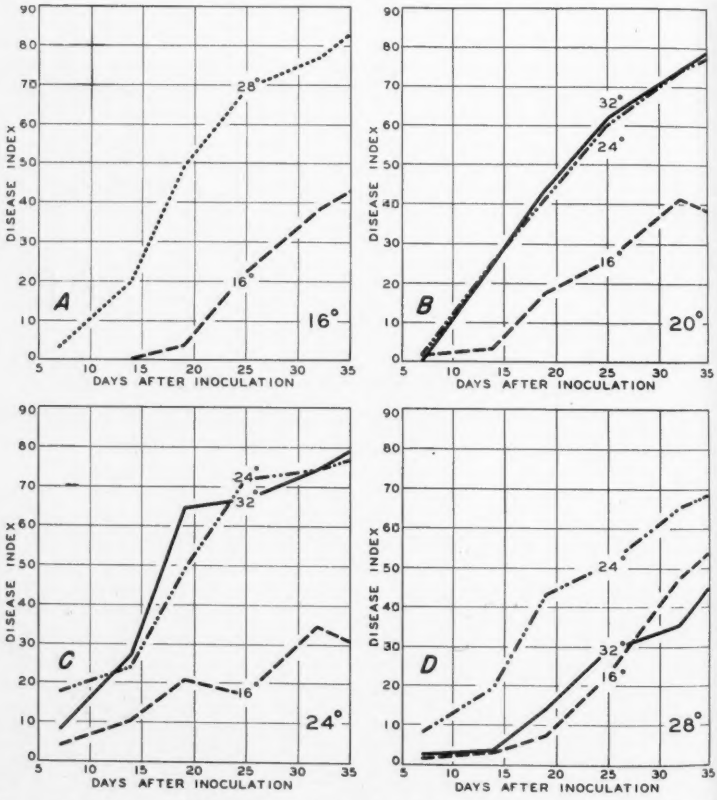

FIGURE 1.—Disease index curves of plants grown at soil temperatures indicated on curves when air temperature was maintained at: *A*, 16°; *B*, 20°; *C*, 24°; *D*, 28°.

The progress of disease is shown in figure 1 and the final disease indices are given in table 2. At 16°, 24°, and 28° air temperatures, maximum disease occurred where soil temperatures were 24° or above. The influence of high soil temperatures upon disease development was most apparent at 16° air, although wide differences occurred at 20° and 24° air. The disease was retarded markedly at 16° soil at each air temperature, maximum development for this soil temperature occurring at 28° air. The disease development was not as great as expected at 32° soil and 28° air but, as stated earlier, the low light intensity in that house influenced the results and the data were not so reliable. Plants developing at favorable soil or air temperature before and after inoculation were affected more severely by the disease than those grown at unfavorable temperatures. In general, therefore, the present results confirmed those secured by Blood (*1*).

PREDISPOSITION BY SOIL TEMPERATURE

In an experiment designed to show the predisposing effect of soil temperature on disease development, plants were grown before infection at various soil temperatures until their effects were exhibited by

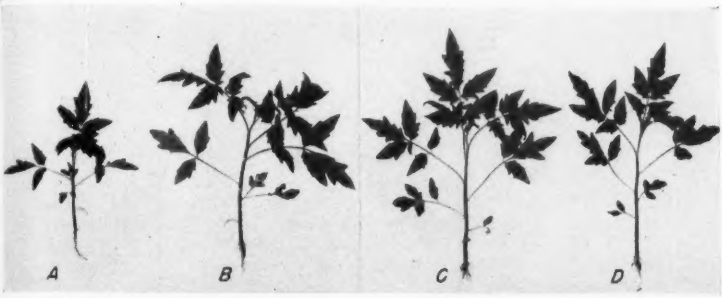

FIGURE 2.—Relative growth of plants predisposed for 31 days at the following soil temperatures: *A*, 16° C.; *B*, 24°; *C*, 28°; *D*, 34°.

differences in plant growth. After inoculation, plants predisposed at different temperatures were placed under uniform conditions so that the effect of predisposition could be expressed. This differs from the usual method of investigation of environal factors, in which the conditions used before inoculation are continued after inoculation. The later method was used by Blood (*1*) and in the experiment reported in the previous section.

In each of 12 crocks, 5 plants were grown at the following soil temperatures: 16°, 24°, 28°, and 34° C. An average air temperature of 20° and an optimum soil moisture for host development were maintained. Average plant weights at the end of 31 days were: at 16°, 2.6 gm.; at 24°, 4.8 gm.; at 28°, 5.0 gm.; at 34°, 4.2 gm. Representative plants are shown in figure 2. The plants were dug and 40 plants from each temperature were inoculated, while 20 plants from each temperature were treated in saline to serve as controls. After resetting in the original soil, the inoculated and uninoculated plants from each predisposition temperature were divided equally between

the 4 soil temperatures, i. e., plants predisposed at each soil temperature were grown after inoculation at soil temperatures of 16°, 24°, 28°, and 34°.

In table 3 are shown the weights of uninoculated plants at the end of the experiment. Plants predisposed at 24° soil weighed as much as or more than those of the other predisposition groups at each postinoculation temperature. Plants predisposed at 16° soil grew very much better at 16° after inoculation than was ordinarily expected and at other postinoculation temperatures they were second to 24° plants in weight. Plants predisposed at 28° soil and 34° soil, although they showed good growth in the predisposing period, failed to adapt themselves to the lowest temperatures sufficiently to show equal growth with those predisposed at low temperatures and transferred to high temperatures after inoculation. Growth at 34° soil before and after inoculation yielded the lowest average weight. When each preinoculation temperature group was considered, the best growth occurred at a postinoculation soil temperature of 28°.

TABLE 3.—*Effect of soil temperature in predisposing tomato plants to disease development and upon growth of the uninoculated host*

Temperature		Average weight uninoculated plants		Disease index at 35 days
Postinoculation	Preinoculation	At time of inoculation	At end of experiment	
°C.	*°C.*	*Grams*	*Grams*	
16	16	2.6	35.6	40.0
	24	4.8	35.6	46.7
	28	5.0	28.6	62.5
	34	4.2	29.0	52.5
24	16	2.6	40.0	68.9
	24	4.8	48.6	48.6
	28	5.0	38.6	80.0
	34	4.2	31.0	80.0
28	16	2.6	45.0	75.0
	24	4.8	52.4	72.0
	28	5.0	42.0	82.9
	34	4.2	41.2	85.0
34	16	2.6	41.5	15.0
	24	4.8	43.0	30.0
	28	5.0	31.2	80.0
	34	4.2	26.0	82.5

The relative progress of the disease in different treatments is shown in figure 3 and the disease indices at the end of the experiment are given in table 3. Insofar as the over-all picture is concerned, the maximum disease development occurred at a postinoculation soil temperature of 28° C. This is in agreement with Blood's results (*1*). However, within each postinoculation treatment, predisposing soil temperatures had a profound influence. For instance, plants predisposed at 24°, which gave maximum growth of the host at each postinoculation temperature, showed less disease than other predisposition groups at each postinoculation temperature. The next level in resistance occurred in plants predisposed at 16° which showed the second best host growth in uninoculated controls. Plants predisposed at 28° and at 34° were most susceptible to the disease at each of the postinoculation temperatures. This is at variance with Blood's con-

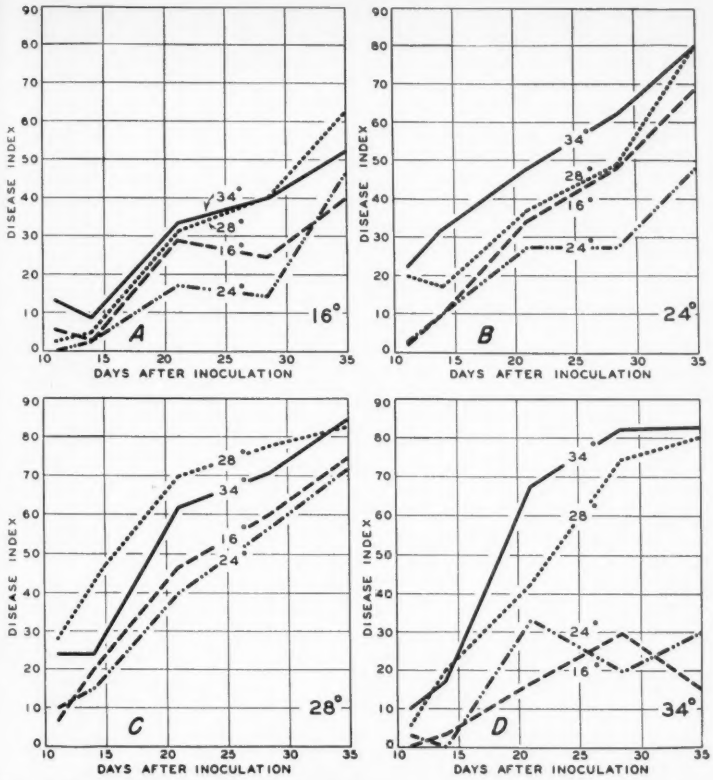

FIGURE 3.—The development of disease symptoms in plants predisposed at soil temperature 16°, 24°, 28°, and 34° C. for 31 days, inoculated, and grown at the following postinoculation temperatures: *A*, 16°; *B*, 24°; *C*, 28°; *D*, 34°.

clusion that optimum conditions for host development are also optimum for disease development (*1*). It is evident that when plants are predisposed at soil temperatures which promote maximum development, and are then inoculated, they are best able to resist disease development at a soil temperature which is optimum for disease development. Moreover, difference in resistance is more striking when they are grown after inoculation at soil temperatures above or below the optimum for the disease.

In plants which were most succulent at the time of inoculation and which grew under suboptimum conditions for the disease after inoculation, the formation of open and unbroken cankers was most frequent. This phase of the disease was most common in plants predisposed at soil temperatures of 28° and 34° and grown after inoculation at 16° and 24°.

In a study of fusarium wilt of tomato, Foster and Walker (*3*) predisposed plants at soil temperatures of 12°, 20°, 28°, and 36° C. and continued plants from each predisposition group at each of the four temperatures after inoculation. Growth of uninoculated plants at the time of inoculation was best at 20° and nearly as good at 28°. The plants predisposed at the two temperatures which promoted best growth succumbed most rapidly to wilt at each postinoculation temperature. From this and the results reported above it appears that predisposing soil temperatures favorable for growth of the tomato make it more susceptible to development of fusarium wilt and less susceptible to development of bacterial canker.

PREDISPOSITION BY AIR TEMPERATURE

In the present investigation the effect of air temperature on predisposition of the host to disease development was studied in a manner similar to that described for soil temperature. Different air temperature treatments were used before infection of the host. Then plants from each treatment were placed under uniform conditions after inoculation so that predisposing differences could be expressed. Sixteen clay pots, 10 plants per pot, were kept for 5 weeks before inoculation at 16°, 20°, 24°, and 28° C. Soil temperatures ranged from 1° to 4° below the respective air temperatures because of the cooling effect of evaporation of water from the soil. Growth of plants at the 4 temperatures at the end of this period is shown in figure 4. The average plant weights were: at 16°, 1.0 gm.; at 20°, 1.8 gm.; at 24°, 4.4 gm.; at 28°, 3.0 gm. After the predisposing period, 120 plants at each temperature were inoculated and 40 were treated in saline to serve as controls. The plants were reset in the same soil and both inoculated and uninoculated plants from each predisposing temperature were divided equally between the 4 air temperatures.

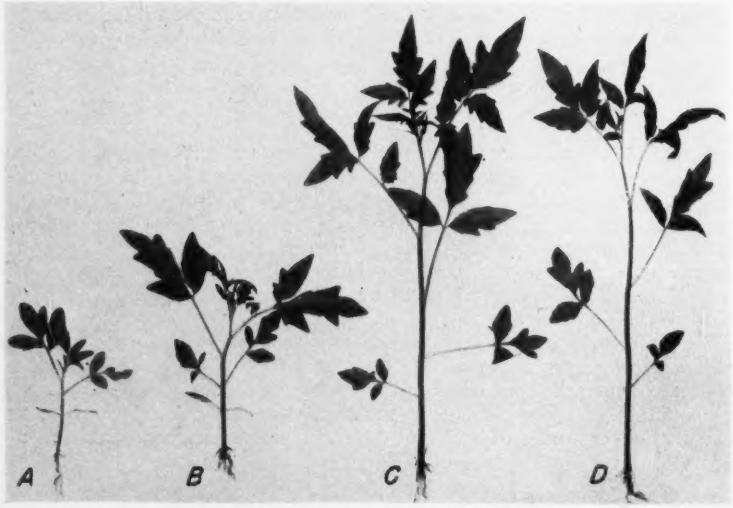

FIGURE 4.—Relative growth of plants predisposed for 35 days at the following air temperatures: *A*, 16° *C.*; *B*, 20°; *C*, 24°; *D*, 28°.

Thus, plants from each predisposition group received postinoculation temperatures of 16°, 20°, 24°, and 28°. This experiment was conducted through November, December, and January, when the lowest light intensity of the year occurred. Because of adjacent glass houses, light intensity in the 28° house was extremely low and plant growth was, therefore poorest at this temperature. As shown in table 4, plants predisposed at 24° grew best at 20°, 24°, and 28° after inoculation, with a maximum at a postinoculation air temperature of 24°. This is at variance with the report of Blood (*1*) who found that 28° was optimum for growth. The discrepancy is probably due to the low light intensity mentioned above. At 16° there was little difference between plants predisposed at 20° and 24°, both groups showing better growth than the other two predisposition groups at this postinoculation temperature.

TABLE 4.—*Effect of air temperature in predisposing tomato plants to disease development and upon growth of the uninoculated host; experiment 1*

Temperature		Average weight uninoculated plants		Disease index at 40 days
Postinoculation	Preinoculation	At time of inoculation	At end of experiment	
°C.	°C.	*Grams*	*Grams*	
16	16	1.0	6.7	30.0
	20	1.8	14.6	28.0
	24	4.4	13.1	23.6
	28	3.0	10.6	37.2
20	16	1.0	12.0	74.1
	20	1.8	17.6	62.8
	24	4.4	18.5	48.0
	28	3.0	15.0	56.3
24	16	1.0	14.3	82.8
	20	1.8	15.7	86.9
	24	4.4	24.0	59.3
	28	3.0	15.5	80.0
28	16	1.0	6.9	82.2
	20	1.8	10.7	86.2
	24	4.4	16.2	85.0
	28	3.0	14.7	89.5

The progress of disease is shown in figure 5 and the final disease indices are given in table 4. The over-all picture of disease at the four air temperatures again agrees with that found by Blood (*1*) in that maximum disease development occurred at the postinoculation temperature of 28° C. and the next greatest progress at 24°. However, within any one postinoculation treatment, differences due to varied predisposing temperatures were apparent. For instance, plants predisposed at 24° showed the greatest resistance to disease at postinoculation temperatures of 16°, 20°, and 24°. At the postinoculation temperature of 28° the results were not reliable because of the reduced light intensity and all plants developed the disease to almost the same extent. Thus, as in predisposition by soil temperature, these results are in contrast to those of Blood (*1*) in that plants placed before inoculation under conditions favorable for growth were more resistant to the disease after inoculation than poorly developed plants.

A second experiment run in a similar manner through December, January, and February when light intensity was improved and with plants at 28° placed so that they received more light, gave essentially

TABLE 5.—*Effect of air temperature in predisposing tomato plants to disease development and upon growth of the uninoculated host; experiment 2*

Temperature		Disease index at indicated number of days after inoculation					Average final weight of uninoculated plants
Post inoculation	Preinoculation	13	18	25	32	37	
°C.	°C.						*Grams*
16	16	0	21.2	16.7	30.7	42.7	11
	20	0	8.4	16.3	30.7	44.4	16
	24	0	15.0	5.0	20.0	24.3	19
	28	0	28.2	29.2	51.2	62.5	12
20	16	0	1.3	49.3	68.7	79.3	17
	20	0.8	5.9	27.7	47.2	53.1	21
	24	0	0	30.4	50.0	56.4	22
	28	2.4	5.4	47.8	70.9	78.2	16
24	16	1.4	21.2	29.0	47.9	62.2	24
	20	.7	13.1	26.7	58.2	63.0	26
	24	.9	7.3	26.1	47.3	58.2	27
	28	20.2	46.5	57.5	84.9	90.9	26
28	16	5.2	32.5	49.3	71.4	80.7	25
	20	4.2	30.0	35.0	66.0	76.7	24
	24	1.5	15.4	20.0	49.2	60.0	30
	28	13.2	47.2	64.8	80.0	82.8	26

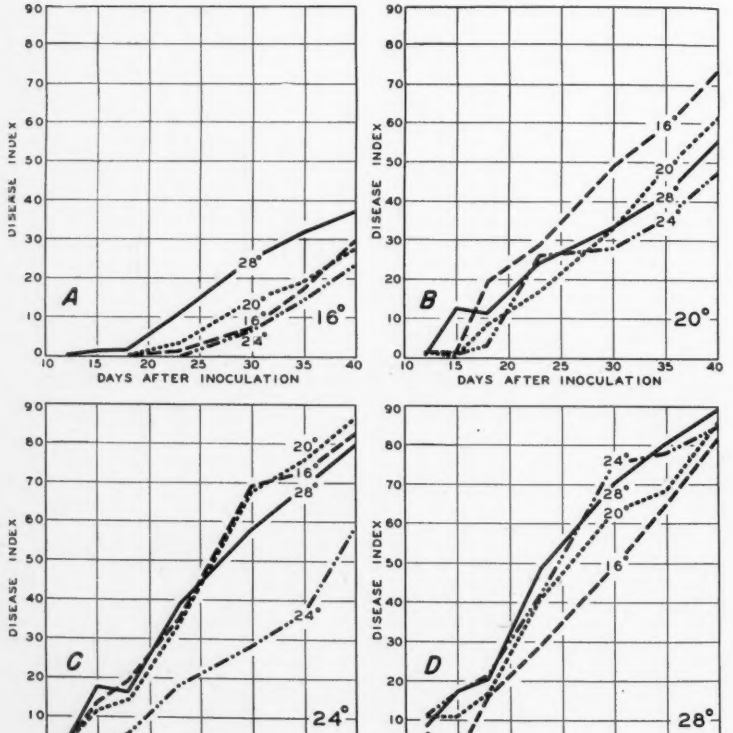

FIGURE 5.—Disease index curves for plants predisposed at air temperatures 16°, 20°, 24° and 28° when grown after inoculation at: *A*, 16°; *B*, 20°; *C*, 24°; *D*, 28° C.

the same results (table 5). Maximum growth before inoculation was obtained at 24°, but as the days became longer postinoculation growth at 28° was equal to that at 24°. The plants retained their original relative order as to size, however, and those predisposed at 24° were the largest at each postinoculation temperature. Maximum disease development occurred again at 28°. However, plants predisposed at 24° showed less susceptibility to the disease at postinoculation temperatures of 16°, 24°, and 28° than those of the other predisposition groups. At 20° the growth of plants predisposed at 20° and 24° was almost the same, as were also their disease indices. Both were less than those of the other two predisposing groups. Even under optimum air temperature conditions for the disease, where light was not so limiting a factor as in the first experiment, the largest plants at the time of inoculation showed least susceptibility to disease.

In a similar series of experiments with fusarum wilt of tomato, Foster and Walker (*3*) secured best preinoculation growth at 28°. They obtained most rapid disease development after inoculation in plants which were largest at the time of inoculation. Thus air temperature, as shown in the previous section with regard to soil temperature, had opposite effects on tomato plants in their predisposition to fusarium wilt and bacterial canker. The largest, most vigorous plants were in both cases the most susceptible to wilt and the least susceptible to canker.

PREDISPOSITION BY SOIL MOISTURE

Blood (*1*) found that the incubation period was longer and the rate of disease progress slower in infected plants growing after inoculation at low soil moisture than in those growing in soil near the optimum in moisture content. He suggested that moisture slightly below the optimum for plant growth was most favorable to the development of the disease.

In this investigation a study was made of the role of soil moisture in predisposing the host to the disease. Plants were grown at high (slightly below 100 percent water-holding capacity), optimum (80 percent water-holding capacity), and low (40 percent water-holding capacity) soil moisture levels in glazed pots at 28° C. air temperature. The type of soil and the moisture levels were approximately the same as those used by Foster and Walker (*3*). Plant weights were estimated and corrected for in the daily adjustment of the moisture content of the soil. Nine pots, with 10 plants each, were predisposed for 28 days at each moisture level. Relative growth at the end of this time is shown in figure 6 *A*, the average plant weights being 1.5 gm. at low, 4.0 gm. at optimum, and 2.0 gm. at high soil moisture. The plants at high moisture (*a*) were spindly and somewhat etiolated; at the optimum level (*b*) they were stocky and vigorous; and at the low level (*c*) they were small, but hardened. The plants were dug, inoculated, and replaced in new compost soil of the same composition as that originally used. A minimum amount of water was supplied for 2 days until the plants recovered partially, and the moisture content was then increased to the optimum and high levels by the addition of water. The soil in the low moisture pots was allowed to dry to the desired level. Twenty inoculated and and 10 uninoculated plants from each predisposition treatment were given postinoculation treatments of low, optimum, and high soil mois-

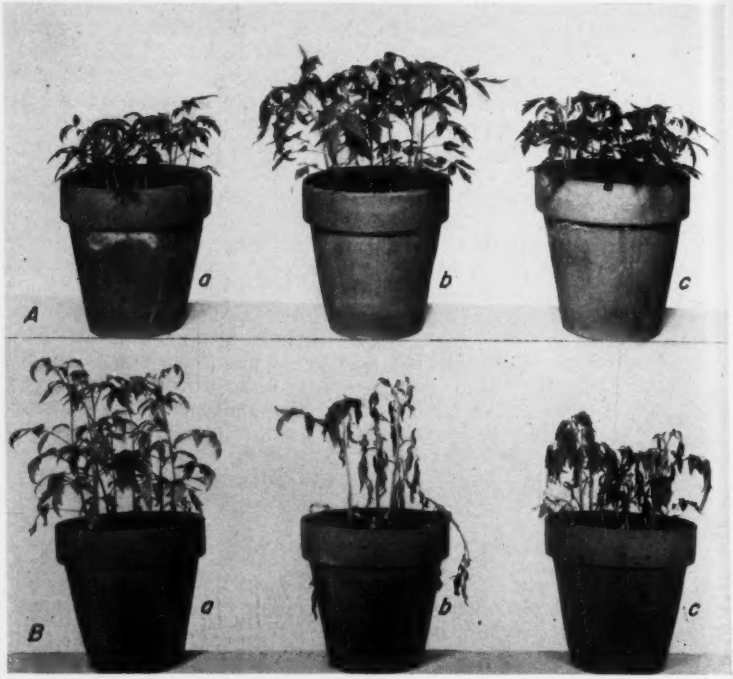

FIGURE 6.—*A*, Comparison of growth of tomato plants predisposed for 28 days at high (*a*), optimum (*b*), and low (*c*) soil moisture levels with air temperature at 28° C.; *B*, the same plants continued at the same moisture levels for 25 days after inoculation.

ture. Thus, 30 plants predisposed at each of the 3 soil moistures were placed at low moisture, 30 at optimum moisture, and 30 remained at the original moisture. From the average weights of control plants (table 6) it may be seen that each predisposition group of plants maintained its respective original rank as to growth throughout the remainder of the experiment.

Disease indices throughout the experiment are recorded in table 6 and those at the end of the experiment are represented graphically in figure 7. In the plants predisposed at optimum moisture, the disease progressed slightly more rapidly after inoculation in the optimum and low moisture groups. The differences between the three predisposition groups, however, was slight. High soil moisture, on the other hand, had a decidedly depressing effect upon disease development after inoculation and the effect was much the same on all three predisposition groups. Those plants predisposed at high moisture and maintained at the same level after inoculation had the lowest final disease index (fig. 6, *B*).

A second experiment run in exactly the same manner under conditions as nearly the same as possible yielded essentially the same results. The main difference was that plants grown at the high moisture level after inoculation failed to show symptoms regardless of the predis-

position treatment until the soil was allowed to dry out. When this happened, plants predisposed at optimum soil moisture showed the first signs of disease.

The chief effect of soil moisture was upon the host-parasite interaction after inoculation. The predisposing effect of soil moisture was not important. This is in contrast with the predisposing effect of air and of soil temperature, in which instances the stockiest and most vigorous plants at inoculation were the most resistant. Although widely different plants were produced at the three soil moisture predisposition levels (fig. 6), the most vigorous were not the most

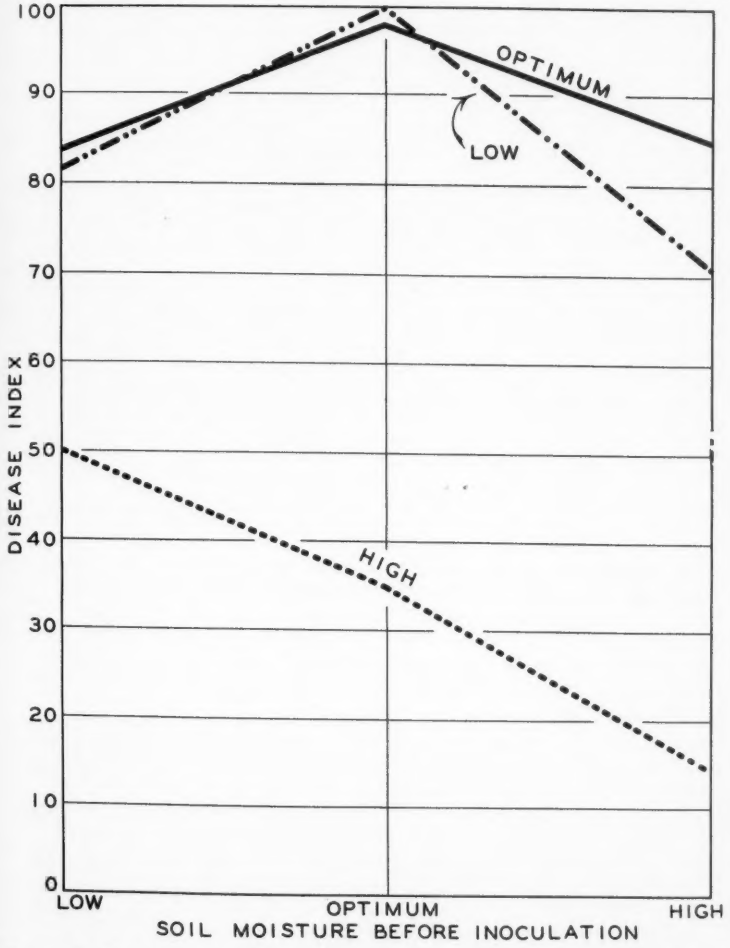

FIGURE 7.—Relation of postinoculation soil moisture level indicated on the respective curves to disease development 29 days after inoculation in plants predisposed at low, optimum, and high soil moisture.

resistant; in fact, they were usually the most susceptible to the disease. Predisposition of tomato at soil moisture levels was not the same for fusarium wilt as for bacterial canker. Foster and Walker (*3*) secured distinct predisposition effects with the former disease. The plants predisposed in dry soil and in wet soil were both decidedly stunted, as in this experiment, but those from dry soil were most susceptible to fusarium wilt.

PREDISPOSITION BY LIGHT INTENSITY

Plants were grown at two light intensities for 30 days before inoculation. A low light intensity was provided by screening with cheesecloth. The light in this case varied from 40 to 700 foot-candles. The normal light of the greenhouse varied concurrently from 100 to 1,600 foot-candles. The experiment was conducted in July and August. After inoculation some plants were left at the light intensity at which they were predisposed and an equal number were changed from one intensity to the other. The progress of the disease in each treatment is shown in table 7. Plants predisposed at low light intensity became diseased more rapidly than those predisposed at normal light intensity regardless of the light intensity after inoculation. This effect is similar to that recorded by Foster and Walker (*3*) for fusarium wilt. Postinoculation intensity was most influential on plants predisposed at low light intensity; those in normal light after inoculation made the fastest growth and the disease developed most rapidly in them.

TABLE 6.—*Effect of soil moisture upon predisposition of tomato plants to disease development and upon growth of the uninoculated host*

Moisture		Disease index at indicated number of days after inoculation							Average weight of control plants	
After inoculation	Before inoculation	9	11	14	17	23	26	29	At time of inoculation	At end of experiment
									Grams	*Grams*
Low	Low	0	4.2	18.9	46.3	68.4	75.8	82.1	1.5	12.2
	Optimum	0	15.4	33.8	41.8	78.2	94.5	100.0	4.0	16.3
	High	0	0	5.3	15.6	38.9	63.2	72.6	2.0	14.0
Optimum	Low	5.9	16.5	27.2	41.2	63.5	58.9	83.5	1.5	16.6
	Optimum	0	17.5	41.2	58.8	91.2	95.0	97.5	4.0	21.0
	High	7.0	14.0	21.1	44.2	67.8	76.8	85.3	2.0	19.0
High	Low	0	1.0	2.0	2.0	18.0	22.0	50.5	1.5	14.3
	Optimum	0	1.7	0	8.3	13.0	25.0	35.0	4.0	18.2
	High	0	0	2.0	2.0	14.0	14.0	14.0	2.0	16.0

TABLE 7.—*Progress of the canker disease and growth of uninoculated controls in plants predisposed at different light intensities*

Light intensity		Average weight uninoculated plants		Disease index at indicated number of days after inoculation		
Before inoculation	After inoculation	At time of inoculation	At end of experiment	12	22	31
		Grams	*Grams*			
Low	Normal	2.1	17.3	19.1	43.5	95.1
Low	Low	2.1	6.4	6.3	15.3	72.9
Normal	Normal	5.9	17.1	6.1	13.0	63.0
Normal	Low	5.9	12.0	3.8	8.5	57.0

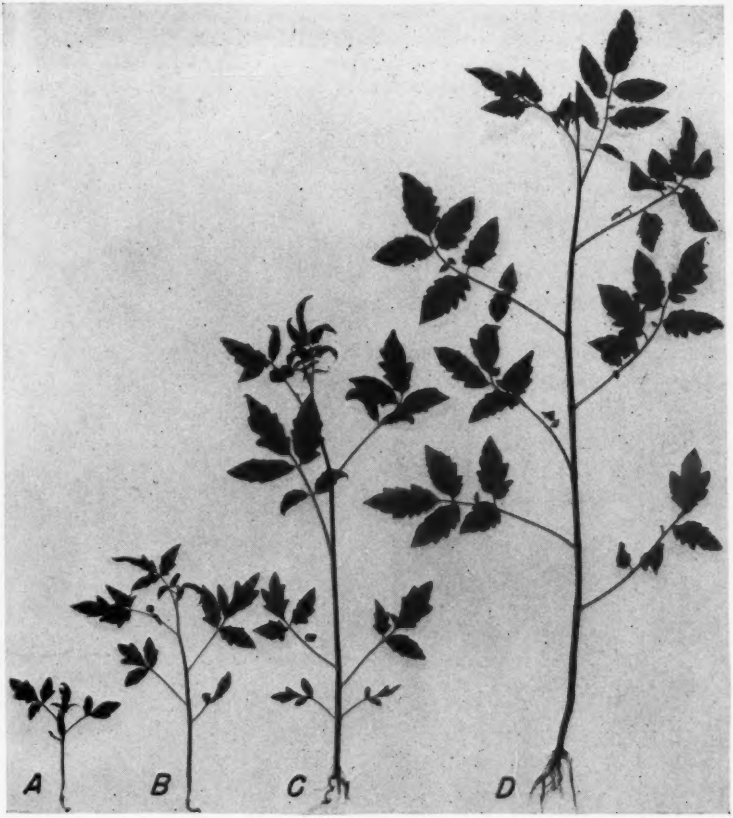

FIGURE 8.—Relative size of tomato plants grown for 33 (*A*), 49 (*B*), 56 (*C*), and 77 (*D*) days, respectively, at 28° C.

AGE OF THE HOST IN RELATION TO PREDISPOSITION

Fifty plants were transplanted into compost soil in flats at 4 successive intervals and were grown at 28° C. for 38, 46, 60, and 76 days, respectively. The average weight of 76-day-old plants was 11.5 gm.; of 60-day-old plants, 4.6 gm.; of 46-day-old-plants, 1.5 gm.; and of 38-day-old plants, 0.2 gm. (fig. 8). Ten plants of each group were used as controls. After inoculuation the plants were reset in new soil in flats. The progress of the disease is shown in figure 9. The disease increased in severity at about the same rate in the 4 age groups, the chief difference being the time which elapsed before symptoms appeared. Thus, the disease was more severe on the younger plants, but the actual rate of development in the older plants after the initial expression of symptoms was just as rapid. Two other experiments conducted in a similar manner with plants of approximately the same ages as those listed above gave similar results. Canker formation was

sparse and there was no difference in frequency between plants inoculated at different ages. Foster and Walker (*3*) likewise found that the age of plant at time of inoculation had little or no effect upon the rate of development of fusarium wilt.

DISCUSSION

The progress of bacterial canker was most rapid at soil and air temperature around 28° C., which was optimum for the growth of the host plant and only slightly below that for the pathogen. In a parallel study (*7*) the concentration of a balanced nutrient solution was found to have an influence also. The rate of disease development increased

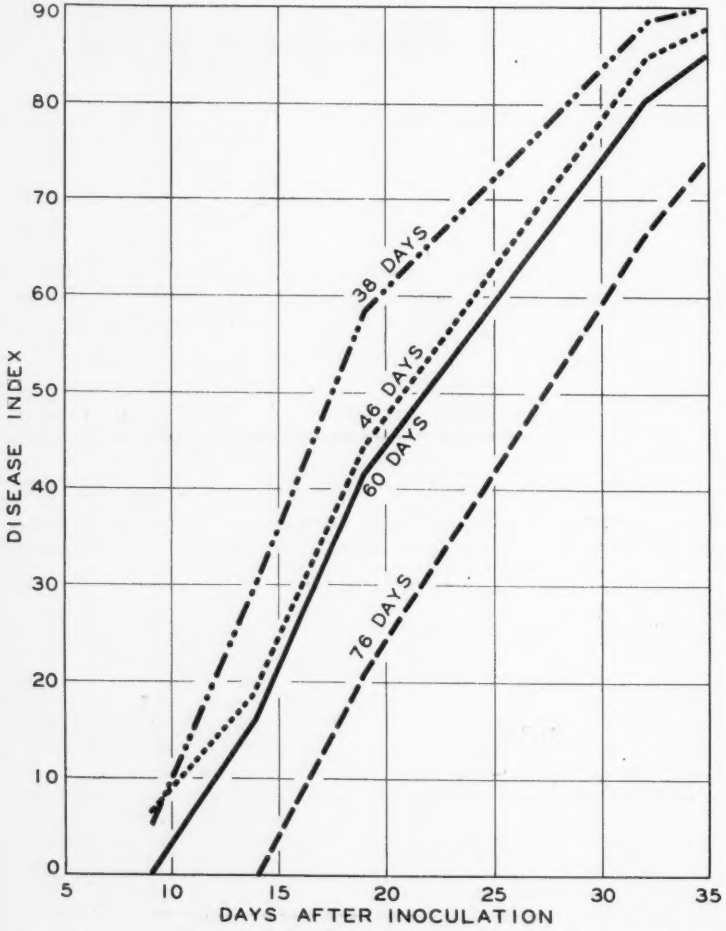

FIGURE 9.—Progress of disease at 28° C. in plants 38, 46, 60, and 76 days old at the time of inoculation.

with increase in concentration up to twice that of the basal solution and somewhat above the optimum for growth. The environment under which the host was grown before inoculation, however, has a measurable influence which can be measured by the rate of disease development after infection. When plants were grown in 24° soil before inoculation, which was near the optimum soil temperature for growth, they were more resistant to disease expression than when predisposed at higher or lower soil temperatures. Likewise, plants grown at 24° air temperature (with a slightly lower soil temperature), again near the optimum for growth, showed measurable resistance as compared with plants predisposed at higher or lower temperatures. On the contrary, when plants were predisposed at optimum soil moisture for a given soil they were more susceptible to disease development than plants predisposed at higher or lower soil moistures for the same soil type. Plants which were predisposed at low light intensity were more susceptible than those grown at relatively higher light intensity.

When these results are compared with those secured by essentially the same methods with the fusarium wilt of the same host (*3*), it is clear that some of the environal factors studied predispose the plants quite differently to the two vascular pathogens. It has already been shown (*5*, *7*) that increase in nutrient concentration after infection enhances bacterial canker and retards fusarium wilt. Predisposition of the plants for 30 days either at the near-optimum soil temperature or near-optimum air temperature increases susceptibility to fusarium wilt and increases resistance to canker as measured by the rate of disease development. Predisposition at low soil moisture increased susceptibility to wilt and optimum soil moisture provided plants most susceptible to canker. Predisposition at low as compared with normal or high light intensity resulted in plants more susceptible to both pathogens. It is obvious that in neither disease was there any correlation between the external appearance of plants as measured by size, weight, or vigor and their susceptibility to either disease.

This investigation opens up questions rather than answers them. It is not out of order to emphasize, however, that in the general field of environment in relation to plant disease the influence of factors upon predisposition of the host cannot be overlooked. It is obvious that the external appearance of the plant is no index to its response in the interaction with the pathogen. It is quite clear that the reactions to two vascular pathogens may be diametrically opposite. If that is so, what variation may be expected with pathogens of greater dissimilarity? Furthermore, plants reduced in growth by dissimilar factors react in opposite fashion to the same pathogen. These superficial observations only emphasize the long recognized but little investigated fact that disease inception and development is bound up with the integrated cellular physiology of the two organisms.

It was pointed out in recent papers (*5*, *6*, *7*) that fusarium vascular pathogens follow a certain pattern in relation to host nutrition different from that of the phloem invader concerned here. It was suggested that this might be explained in part by the fact that the phloem invader showed a distinct requirement for elaborated nitrogen sources in pure culture (*4*), while the other is a xylem invader which thrives on inorganic nitrogenous compounds. It was suggested that the location of the organisms in the host tissue with respect to source

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and availability of foods might account for differences in their activity as pathogens. There is much less to justify such a theory in explanation of the influence of predisposing factors. It is possible, however, that some clues may be secured by a micro- or macrobiochemical study of plants predisposed in different ways.

SUMMARY

Tomato plants of the variety Bonny Best were predisposed to various environments for a 30-day period before inoculation with the canker organism (*Corynebacterium michiganense* (Sm.) H. L. Jensen).

When plants were predisposed at a soil temperature of 24° C. and an air temperature of 20° they succumbed to the disease less rapidly than plants predisposed at higher or lower soil temperatures.

In plants which were predisposed at an air temperature of 24° C. and a soil temperature slightly below that point the disease progressed less rapidly than in plants predisposed at higher or lower air temperatures.

Plants grown at optimum soil moisture succumbed more rapidly than those predisposed at higher or lower soil moistures for a given soil.

Plants predisposed at low light intensity became diseased more rapidly than those predisposed at relatively higher light intensity.

In plants inoculated at different ages the disease progressed at the same rate but symptoms appeared less promptly in the oldest than in the youngest plants.

The predisposing effect of soil temperature, air temperature, and soil moisture on the host plant in its reaction to the canker pathogen was quite different from and almost diametrically opposite to that of the same factors on the host in its reaction to the fusarium wilt organism.

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POLLEN COMPATIBILITY IN ASCLEPIAS SYRIACA[1]

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INTRODUCTION

During World War II it was suggested that common milkweed (*Asclepias syriaca* L.) be brought under cultivation on a commercial scale for the production of its seed floss. Should such a project be undertaken, it would be necessary to develop high-yielding strains since the low yield of floss is one of the most serious obstacles in the way of the adaption of *A. syriaca* as a successful crop plant. Within this species have been found strains with various pod types that are associated with different yields and characteristics of floss (*12, 16, 17*)[3] and that would be a basis for the development of improved stocks.

Before genetic studies directed toward the development of improved stocks could be begun it would be necessary to know how to hand-pollinate the intricate flowers of *Asclepias syriaca* and how much self-incompatibility there is in the species. The present paper describes a successful method of hand-pollination, reports the results of a large number of different types of crosses made to determine the degree of incompatibility within the species, and gives miscellaneous observations on open pollinations.

MATERIALS

In the course of the studies milkweed plants from several sources within Michigan were used. A natural stand on which considerable work was done was located on the Toma farm in western Washtenaw County. A second stand, from which a variety of pod types was obtained, was in a court of the University of Michigan Botanical Gardens, where it had been maintained for many years. A third was established as a plantation in the Botanical Gardens and consisted of

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[2] The writers are indebted to Walter F. Kleinschmidt, head gardener of the University of Michigan Botanical Gardens, for assistance in perfecting a successful method of hand-pollination of the intricate *Asclepias* flowers, and to Mary Bernard Klepper, research assistant, Botany Department, University of Michigan, for assistance in the pollination work and in the preparation of figure 1.

[3] Italic numbers in parentheses refer to Literature Cited, p. 198.

plants from numerous localities in Washtenaw County and adjoining parts of Livingston County and from Emmet County, in the northern part of the State. Greenhouse experiments were also carried on during the winter with material from the first two sites mentioned.

METHOD OF HAND-POLLINATION

As is well known, the asclepiad flower is highly specialized for entomophily, exceeding even the orchids in this respect. It was the subject of morphological and biological investigations by nineteenth century botanists such as Brongniart (*1*), Brown (*2*), Schacht (*15*), Payer (*11, pp. 567–570*), Delpino (*5*), Hildebrand (*7*), and Corry (*3, 4*). Recently Woodson (*18*) and Moore (*9*) also surveyed briefly the principal features of the flower. Although these investigators published excellent descriptions and illustrations of the flower, to a great many botanists its structure and exact method of pollination still remain obscure.

For the present paper it is sufficient to point out that access to the functional stigmatic surfaces is reached by five fissures each bearing a pair of coriaceous protruding flaps considered to be the margins of the anther sacs (fig. 1, *a*). The pollinia are lodged in pockets on either side of the fissures and are joined in pairs by means of the ascending translator arms, which are joined above the fissure to a hollow, black, grooved clip. As pointed out by Corry (*3*), Frye (*6*), and others, the pair of pollinia thus straddling a fissure (fig. 1, *b*, *b'*) are derived from adjacent anthers.

Each pollinium is a paddle-shaped structure (fig. 1, *c*), the two edges of whose broader portion differ in their degree of convexity. It is from the more convex edge that the pollen tubes emerge.

The process of pollination consists of extracting pollinia from their pockets and placing them in the stigmatic cavities. It was found that this very arduous work was greatly facilitated by the use of a headband magnifier and forceps with very fine, sharp points.

After the pollinia are extracted from their pockets, they may be used directly in making a pollination or placed temporarily on filter paper in a petri dish. If they are used directly, it is necessary to pause a few seconds after extraction and before insertion to allow them, by the curious twisting of the translator arms, to become oriented approximately at right angles to the plane they occupied originally (*4*). They are thus brought into a position to be inserted with relative ease through the fissure and into the stigmatic cavity.

Pollinia may be stored as long as 45 days at 4° C. and maintain their viability. A slightly higher percentage of germination (100 percent as compared with 95 percent) was obtained from pollinia stored under like conditions but left in flowers stored in petri dishes on slightly moistened filter paper.

The paired pollinia may or may not be separated in making a pollination. A separated pollinium did not appear to be any easier to insert than an individual one of an intact pair or vice versa.

In the process of transference, a pollinium is grasped at its narrower end or by the translator arm and held with its more convex edge foremost. The strongly convex edge is inserted into the more open, flaring base of the cleft of the flower (fig. 1, *a*) lightly held in an inverted

position. It is then drawn slightly downward (upward as the drawing is oriented), tilted inward, and released (fig. 1, *c*, *c'*). Difficulty in inserting a pollinium may be due to the fact that the fissure, particularly in a newly opened flower, may be more or less tightly closed.

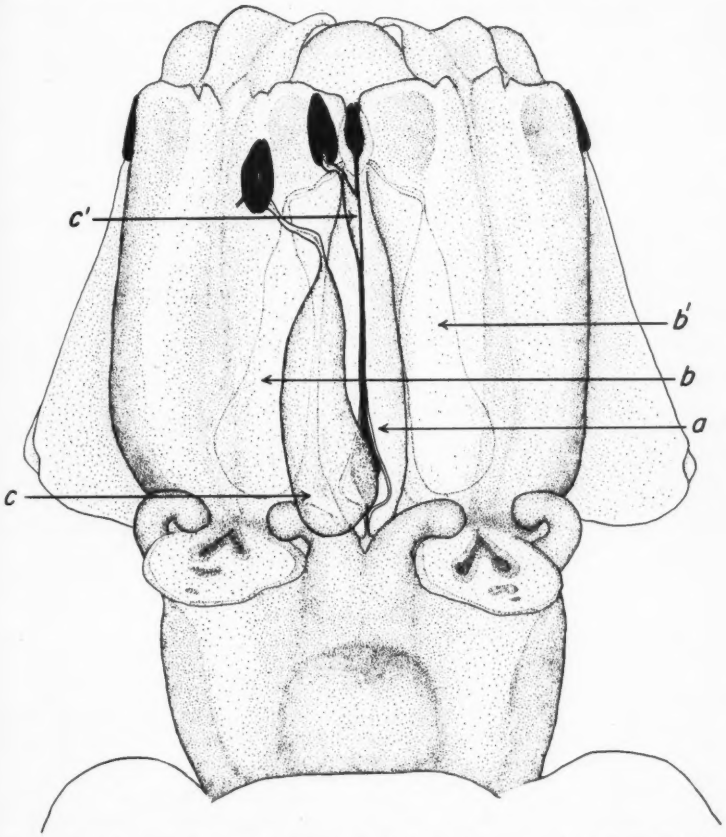

FIGURE 1.—Flower of *Asclepias syriaca* with hoods removed to show relative positions of the pollinia in place in the anther sacs (*b*, *b'*), the flap (*a*) which borders one side of the cleft, or fissure, and the stages in the insertion of the pollinium into the cleft (*c*, *c'*).

For successful pollination it is essential that the more convex edge of a pollinium contact the stigmatic surface hidden within the cleft, for, as mentioned earlier, it is from this region of the pollinium alone that the pollen tubes emerge. Some unsuccessful pollinations result from incorrect orientation of the pollinia and from injury of the flower by rough handling. Others, however, are due to the failure of the pollinia to germinate.

To increase the chances that a pollination would be successful, usually pollinia were placed in two adjacent stigmatic pockets. Moore (*9*) found that for successful pollinations it is necessary that pollinia be placed in two adjacent pockets. He was of the opinion that one pollinium cannot supply with pollen tubes a sufficient number of the ovules in one ovary to insure the setting of fruit. Since the writers used two pollinia in making most pollinations, their data neither confirm nor disprove Moore's views. It is probable, however, that the necessity for using more than one pollinium was due to the improper orientation of a single pollinium when it was inserted. Nowhere does Moore mention the fact that pollinia germinate only on the more convex surface. Moreover, some of the writers' observations on sectioned material seem to indicate that a pollination with only one pollinium might be successful. Sections through ovaries that were known to have received pollen tubes from only one pollinium showed that nearly every ovule had received a pollen tube.

In making the pollinations routine precautions were observed. Inflorescences in the pink-bud stage were tightly bagged until ready for pollination, and after pollination they were bagged again until the flowers had withered. All except ants and smaller insects were thus excluded from the flowers. There was no evidence which indicated that even large ants were capable of transferring the pollinia.

Usually 10, occasionally 15 to 20, flowers were pollinated in 1 umbel. These were marked by tying a colored thread around the pedicel, the color of the thread indicating the source of the pollen. Pollinated flowers were examined daily, about half of the withered flowers and all of the withering young fruits being brought into the laboratory for examination. Some flowers and fruits were preserved and later embedded in paraffin and sectioned.

COMPATIBILITY TESTS

Very little save general statements could be found in the literature available to the writers at the time the work was begun with respect to the problem of compatibility within *Asclepias syriaca*. The limited observations of Delpino (*5*), Hildebrand (*7*), and Corry (*4*) had pointed uniformly to the complete incompatibility of *A. syriaca* to its own pollen. Plotnikova (*14*), however, had reported a small but variable degree of self-fertility. Since these observations were on plants growing in Europe and some of the data were derived from few plants, it seemed desirable to study compatibility in large numbers of native American wild plants.

Accordingly, pollinations were made with pollen from the following sources: Same flower, different flower of same umbel, flowers of different umbels of same plant, flowers of different plants of same clone, and flowers of plants from a distant locality or of a clone known to produce a different type of pod. The results of these pollinations are given in table 1. In addition, many reciprocal crosses were made with the same results.

In all, 2,794 flowers on about 100 different plants were hand-pollinated with pollinia from different sources. Six hundred and

TABLE 1.—*Mature fruits of Asclepias syriaca obtained from flowers hand-pollinated with pollen from 5 sources*

Source of pollen and location of plants pollinated	Flowers		
	Pollinated	Setting fruits that matured	
	Number	*Number*	*Percent*
Same flower:			
Greenhouse	60	0	0
Botanical Gardens	113	0	0
Toma farm	10	0	0
Total	183	0	0
Different flower of same umbel:			
Greenhouse	22	0	0
Botanical Gardens	101	1	[1] 1.00
Toma farm	10	0	0
Total	133	1	.75
Different umbel of same plant:			
Greenhouse	41	0	0
Botanical Gardens	220	0	0
Toma farm	110	0	0
Total	371	0	0
Different plant of same clone:			
Greenhouse	5	0	0
Botanical Gardens	442	1	[2] .23
Toma farm	220	0	0
Total	667	1	.15
Plant from distant locality or from clone known to produce a different type of pod:			
Greenhouse	591	51	8.63
Botanical Gardens	729	80	10.97
Toma farm	120	18	15.00
Total	1,440	149	10.35

[1] A bee was caught in the meshes of the protecting cheesecloth bag directly over the pollinated flower that developed a fruit.
[2] Pod contained many abnormally shaped and aborted seeds.

eighty-seven pollinations involving the use of pollinia from the same plant resulted in the formation of only a single fruit, almost certainly the result of the accidental introduction of foreign pollen by a bee. Of the 667 flowers pollinated with pollinia from different plants of the same clone, only a single pod reached partial maturity. This one could not be considered normal, however, for, though it was about as long as other pods from the same clone, it was only about half as plump. Many of its seeds were aborted and undersized, although most had formed a tuft of fibers. The remaining 1,440 flowers were all pollinated with pollinia from distant plants or plants known to produce different types of pods. Approximately 10 percent of these flowers formed fully mature fruits. Thus, it has been shown conclusively for at least the particular plants studied that an individual plant of *Asclepias syriaca* is self-incompatible and cannot form mature fruits if self-pollinated; with rare exceptions this incompatibility extends to plants of the same clone. In recent studies carried on in Canada, Moore (*9*) found that self-pollination of *A. syriaca* was always unsuccessful. On the other hand, Stevens (*17*), who also experimented recently with Ameri-

can-grown plants, observed that self-pollination may sometimes be effective, thus confirming the work of Plotnikova (*14*). The variations in degree of self-fertility that have been found may possibly be due in part to differences in the pollination techniques employed, but it is also possible that they represent real differences in the plants or populations studied. A completely satisfactory explanation would require more extensive investigations than have been undertaken.

Successful pollination with compatible pollen brings about certain very obvious changes in the pollinated flowers: namely, a thickening and recurving of the pedicel, an enlargement of the ovary, and, perhaps most striking, the appearance of pubescence on the ovary. Incompatible pollen is also capable of bringing about similar though somewhat weaker responses.

About half of the hand-pollinated flowers were examined after they had withered for two of these postpollination changes, namely development of pubescence and enlargement as measured by increase in length (table 2). In about 40 percent of the flowers examined, regardless of the source of the pollen, pubescence had developed on at least one of the ovaries (see also table 3). In the case of flowers pollinated with pollinia from the same plant (table 2), the pubescent ovaries had elongated very little, the majority not exceeding 5 mm. in length. Four ovaries were longer than 5 mm. but did not exceed 10 mm., and their pedicels had thickened a little and showed a slight curvature. In flowers pollinated with pollinia from another plant of the same clone, the majority of the pubescent ovaries were likewise not longer than 5 mm. In this group, however, the percentage of ovaries longer than 5 mm. was greater than in the preceding groups. One of these had attained a length of 11.8 mm. All flowers with pubescent, decidedly enlarged ovaries had distinctly thickened and recurved pedicels. Pollination with pollen assumed to be compatible, that is, pollen from a distant locality or from a clone known to produce a different type of pod, as might be expected, resulted in the production of many ovaries exceeding 5 mm. in length. Of those failing to become fully mature fruits, the great majority had ceased growth and had begun to wither before they were 25 mm. long and none became longer than 32.8 mm.

Microscopic studies of the preserved withered ovaries of flowers pollinated with pollen from the same flower, umbel, or plant showed that the pollen tubes developed normally, entered the ovules, discharged their nuclei into the embryo sac, and effected fertilization. Coincident with these activities, pubescence developed on the ovaries. Initial enlargement of the ovules may occur, but it soon stops, for in pistils fixed 6 days after an incompatible pollination (4 days after fertilization) the ovules had lost most of their capacity for staining and were definitely shrunken. The embryo sacs, however, may remain turgid somewhat longer. Although the endosperm nuclei may divide, the total number of endosperm nuclei was distinctly less than in ovules of the same age but pollinated with compatible pollen. A cellular condition of the endosperm was observed in one instance after an incompatible pollination. No case of the division of the zygote was found. It was noted that the hairs on the ovary wall, while two to four cells long, were not so well developed as on ovaries of the same age pollinated with compatible pollen. In only one case was seed-

hair development noted, namely, in an ovary fixed 28 days after pollination and with a length of 10 mm. The hairs, however, were only 0.19 mm. in length. None of the ovules observed exhibited the extreme hyperplastic condition which Moore (*8*) described for ovules from aborted pods.

TABLE 2.—*Ovaries that became pubescent and increased in length, based on 1,342 withered flowers or fruits representing hand-pollinations with pollen from 5 different sources*

Source of pollen and location of plants pollinated	Pollinated flowers examined	Pollinated flowers producing pubescent ovaries becoming—			
		Less than 5 mm. long		More than 5. mm. long	
	Number	*Number*	*Percent*	*Number*	*Percent*
Same flower:					
Greenhouse	32	0	0	1	3.1
Botanical Gardens	103	51	49.5	0	0
Total	135	51	37.8	1	.7
Different flower of the same umbel:					
Greenhouse	10	6	60.0	0	0
Botanical Gardens	69	32	46.4	2	2.9
Total	79	38	48.1	2	2.5
Different umbel of the same plant:					
Greenhouse	17	1	5.9	0	0
Botanical Gardens	148	52	35.1	1	.7
Total	165	53	32.1	1	.6
Different plant of the same clone (Botanical Gardens)	357	160	44.8	22	6.2
Plant from distant locality or from clone known to produce a different type of pod:					
Greenhouse	254	78	30.7	44	17.3
Botanical Gardens	352	75	21.3	60	17.0
Total	606	153	25.2	104	17.2
Total from all sources	1,342	455	33.9	130	9.7

Pollinations involving two different plants of the same clone resulted in somewhat more pronounced reactions than pollinations with pollen from the same plant. The ovules in which fertilization had taken place were enlarged to a greater degree, the hairs on the ovary wall were longer, and there were numerous instances in which the endosperm had become cellular. In one ovary which had attained a length of 11.8 mm. slender, somewhat abnormal floss had been formed. As in those ovaries whose development had been stimulated by pollen from the same plant, no zygote was found to have divided and no ovules had developed into what might be considered a hyperplastic condition.

OBSERVATIONS ON OPEN-POLLINATED UMBELS

All investigators of *Asclepias syriaca* have commented on the low yields of mature fruits as contrasted with the large number of flowers produced. Since good yields are of vital importance in attempts to develop *A. syriaca* as a crop plant, any information bearing on the reason for the low yields seems of importance. It might be supposed that in such a highly specialized type of flower, failure of the insects to

accomplish pollination would be an obvious cause of low fruit production. To obtain information on this point, 30 open-pollinated umbels were selected at random for examination from different wild stands of milkweed. These inflorescences had all passed their period of flowering, were distinctly faded, and were no longer attractive to insect pollinators. Table 3 gives a summary of the results of an examination of 1,275 flowers from 30 open-pollinated umbels to determine the percentages of flowers pollinated, of flowers having at least 1 germinated pollinium in the stigmatic cavities, and of flowers having at least 1 ovary pubescent. It should be borne in mind, however, that either compatible or incompatible pollen may cause initiation of pubescence.

TABLE 3.—*Effectiveness of open pollination of 30 umbels of Asclepias syriaca selected at random from wild stands when they were no longer attractive to insect pollinators* [1]

Umbel No.	Flowers per umbel	Flowers pollinated	Flowers with at least 1 germinated pollinium	Flowers with at least 1 ovary pubescent
	Number	*Percent*	*Percent*	*Percent*
1	47	40.4	40.0	25.5
2	14	71.4	64.3	64.3
3	40	52.5	52.5	47.4
4	28	50.0	46.4	42.8
5	57	59.6	42.1	19.3
6	74	70.3	66.3	32.4
7	21	85.7	71.4	42.8
8	85	77.6	75.2	56.5
9	33	18.2	18.2	9.1
10	23	69.5	69.5	60.8
11	39	38.2	30.8	25.7
12	42	47.6	47.6	45.2
13	46	41.3	39.1	39.1
14	37	27.0	24.3	21.6
15	40	82.5	67.5	7.5
16	41	46.3	46.3	4.9
17	45	95.6	93.3	33.3
18	67	77.6	68.7	44.8
19	46	97.8	97.8	50.0
20	60	63.3	56.7	38.3
21	19	78.9	68.4	10.5
22	55	76.3	76.3	27.2
23	26	65.3	65.3	23.0
24	35	80.0	80.0	51.5
25	21	95.2	95.2	85.7
26	55	30.9	23.6	16.3
27	46	41.3	41.3	30.4
28	54	66.7	64.8	64.8
29	40	90.0	87.5	62.5
30	39	97.4	97.4	79.4
Average	42.5	64.5	60.6	38.8

[1] The difference between columns 3 and 4 is the percentage of flowers that were pollinated but in which pollinia did not germinate.

The number of flowers in the various umbels ranged from 14 to 85 (table 3). The percentage of flowers that were pollinated (pollinia in at least 1 of the stigmatic cavities) ranged from 18.2 to 97.8 percent, with an average of 64.5 percent for all umbels. Moore (*10*) found the average percentage per umbel to be 95.3 and Plotnikova (*13*) 98 to 99. These percentages are not comparable with those reported by Stevens (*17*), for his figures are based on the percentage of stigmatic cavities pollinated rather than the percentage of flowers pollinated.

The differences in the percentages of flowers pollinated reported by the writers, by Moore (*10*), and by Plotnikova (*13*) are undoubtedly

due to differences in insect activity in the localities where the plants were grown. Plotnikova's (*13*) plants were grown in the vicinity of beehives, which would account for the nearly 100 percent pollination reported.

Since there are 5 stigmatic cavities into which pollinia can be inserted, the number of these in each of the open-pollinated flowers of the 30 umbels was determined. The results were as follows:

Stigmatic cavities per flower containing pollinia (number):	*Flowers pollinated (percent)*
1	56. 8
2	25. 5
3	11. 5
4	4. 9
5	1. 3

The writers' finding that less than a quarter of the flowers had 3 cavities or more bearing pollinia contrasts with Moore's report (*10*) that the percentage of flowers with 3 to 5 cavities pollinated greatly exceeded the percentage with only 1 or 2 pollinated. The discrepancy in the observations can probably be explained by greater insect activity in the Canadian than in the Michigan localities.

All the pollinia in the stigmatic cavities do not germinate. For the 30 umbels studied, the percentage of flowers having at least 1 germinated pollinium ranged from 18.2 to 97.8 percent, with an average of 60.6 percent. Of the pollinated flowers 94 percent bore at least 1 germinated pollinium; and of all the pollinia observed in the stigmatic cavities 86.6 percent had produced pollen tubes. Since pollinia separated from any floral structure will germinate in sugar solution, either the ungerminated ones had been very recently placed in the cavities—an unlikely assumption because of the condition of the flowers—or they had lost their vitality.

Apparently germination of the pollinia does not necessarily insure a pronounced visible response on the part of the ovary. The percentage of flowers per umbel with at least 1 ovary pubescent ranged from 4.9 to 85.7 percent, with an average of 38.8 percent for the 30 umbels (table 3). This means that only about 64 percent of the flowers with germinated pollinia developed pubescent ovaries.

No completely satisfactory explanation for this low percentage has been found, but failure of pollen tubes to penetrate the ovary and effect fertilization may be a partial explanation. Variations in the extent of pollen-tube penetration were not studied in detail. It was observed in the hand-pollinated material, however, that the extent of the pollen-tube growth may vary. In some instances the tubes had barely penetrated the stigmatic area. In other cases a few tubes had entered the stylar tissue but had not proceeded farther. Microscopic observations indicated that, although the presence of pollen tubes in the style only was sufficient to initiate a small amount of protrusion of some of the epidermal cells of the ovary wall, macroscopically visible pubescence formed only after fertilization.

From these observations it is evident, as observed and commented upon by others, that the lack of pollination is not responsible for the poor setting of fruit in *Asclepias syriaca*. Undoubtedly a large number of the flowers are pollinated with incompatible pollen.

It will be recalled, however, (table 1) that of the large number of

flowers hand-pollinated with what was considered to be compatible pollen, that is, pollen from a distant locality or from a clone known to produce a different type of pod, only about 10 percent developed mature fruit. Of the remaining 90 percent some faded and died without giving any evidence of a reaction to pollination (table 2). In others the ovaries began development, but later aborted. Of the 606 withered ovaries and fruits examined, which represented 606 flowers pollinated with supposedly compatible pollen, 25.2 percent had developed pubescence but had ceased to enlarge before they were more than 5 mm. in length; 17.2 percent, however, had become longer than 5 mm. before they aborted (table 2). Moore (*10*), in a study of pod yield of Canadian-grown plants of *Asclepias syriaca*, found an early flower drop generally of 80 to 85 percent. Since he did not take the development of pubescence into account, it is possible that some flowers whose ovaries had become pubescent but only slightly enlarged were included in the count.

Moore found, however, that of 565 flowers examined 38, or 6.7 percent,[4] had aborted but enlarged ovaries. This value is not comparable with the 17.2 percent reported by the writers, for it is based on observations made on umbels open to insect pollination, whereas the writers' value is based on a group of withered flowers that had been pollinated with supposedly compatible pollen. However, the results of both experiments show, as do the observations of Plotnikova (*13*), that in only a small percentage of the flowers of an umbel does considerable enlargement of the ovary take place and that only a few of these enlarged ovaries develop into mature pods.

Both Moore (*10*) and Plotnikova (*13*) stated that pods may cease to develop at any stage, and the writers' observations also indicate that this is true. Plotnikova (*13*) added, however, that the pods that survive the early stage of development only rarely perish later. This has been confirmed by the writers, for though they made no extensive measurements or counts, they found that as a rule the aborted pods are very much smaller than those of mature size in the same umbel.

The abortion of fruit could possibly be explained in part at least by an insufficient food supply (*8*). There must, however, be many other factors involved. It seems probable that the factors responsible for the few failures of considerably enlarged ovaries to reach complete maturity are distinct from those affecting the smaller ones.

Another possible yield factor is the development of twin fruits. The milkweed flower possesses two separate carpels each of which is potentially capable of producing a mature, well-developed pod. Examples of twin pods are, however, relatively few (*18*).

The development of both carpels is dependent, in the first place, upon the number and arrangement of the pockets containing germinated pollinia. It will be recalled that the five stigmatic cavities are located in a ring around the two carpels and that there are five corresponding but restricted receptive regions of the stigma (*18*). Corry (*4*) observed that the numerous pollen tubes from a single pollinium entered the same carpel and, in describing the penetration of a skein of pollen tubes from the stigmatic cavity in which the pollinium is situated, stated (*4, p. 202*) that it travels " . . . in a direct radial

[4] Value calculated from data presented by Moore (*10*).

line . . . till it arrives at the point of union of the style-table with the apices of the two styles." Because of the distribution of the five stigmatic areas between the two styles, it would be possible, as pointed out by Woodson (*18*), for pollen tubes penetrating three stigmatic areas to result in the development of only one carpel or for those penetrating only two areas to bring about the development of both carpels. Thus, for both carpels to develop, at least two pollinia are necessary, and they must be in stigmatic cavities so situated that pollen tubes from one pollinium will be directed toward one carpel and those from another toward the second carpel.

Among the 30 open-pollinated umbels studied by the writers, by far the greater number of flowers had only a single cavity bearing a germinated pollinium and in none of these had both ovaries reacted, that is, become pubescent (table 4), indicating that the pollen tubes were not distributed between the 2 carpels. This behavior of the pollen tubes was further substantiated by observations on the many hand-pollinated flowers, particularly those sectioned. A single instance was found, however, in which the pollen tubes from a single pollinium were divided between the 2 styles.

With an increase in the number of stigmatic cavities containing germinated pollinia, there was an increase in the percentages of flowers with both ovaries pubescent (table 4). It was observed, however, that of those flowers in which there were two adjacent cavities bearing germinated pollinia, 18.5 percent had both ovaries pubescent, whereas when the two cavities bearing germinated pollinia were separated by an empty one, the percentage was more than doubled (39.8 percent). These percentages can be explained by the fact that the pollen tubes from any two cavities will lead to both styles a greater percentage of the time if the two cavities are separated by another one than if they are adjacent. Skeins of pollen tubes from pollinia in adjacent cavities directed toward the same style apparently do not interfere with one another for both have been observed to enter.

In the writers' examination of 1,992 mature fruits, only 5.4 percent were found to be double, or "twined." Moore (*10*), however, found the percentages to vary from 9.5 to 24.4 for 11 different collections examined. Since he found many more flowers with 3 or more stigmatic cavities containing pollinia than were found among the umbels examined from the Michigan plants, it would be expected that also a higher percentage of the flowers would develop double fruits.

TABLE 4.—*Distribution of pubescent ovaries in flowers bearing germinated pollinia in different numbers of their stigmatic cavities of 30 open-pollinated umbels of Asclepias syriaca*

Stigmatic cavities per flower containing germinated pollinia (number)	Flowers examined	Flowers having pubescence on—		
		Neither ovary	1 ovary	Both ovaries
	Number	*Percent*	*Percent*	*Percent*
1	390	44.6	55.4	0
2:				
Adjacent	65	30.7	50.8	18.5
With empty stigmatic cavity between	88	31.8	28.4	39.8
3	83	31.3	28.9	39.8
4	36	16.7	11.1	72.2
5	11	0	9.1	90.9

SUMMARY

Before successful commercial cultivation of *Asclepias syriaca* as a floss-producing plant can be accomplished, it will be necessary to develop strains whose yield of floss is higher than that commonly encountered among the wild stands. It is important, therefore, to know how to hand-pollinate the intricate flowers of this plant and to determine with certainty the situation within the species with regard to self-incompatibility.

A successful method of hand-pollinating the asclepiad flower is described, and the importance of inserting the pollinia into the stigmatic cavities so that their more convex edges, that is, the edges from which the pollen tubes emerge, are directed inwardly is pointed out. By this method a series of pollinations were made with pollen from five different sources: namely, the same flower, a different flower of the same umbel, flowers of different umbels of the same plant, flowers of different plants of the same clone, and flowers of distant plants or of clones known to produce pods of a different type.

Only flowers that were pollinated with pollen from a distant plant or from a clone known to produce pods of a different type formed well-developed, mature fruits, establishing complete self-incompatibility as far as the development of mature fruits is concerned at least for the plants tested.

Pollen incapable of producing mature fruits may, however, penetrate the ovary and effect fertilization. This results in the development of a macroscopically visible pubescence on the ovary wall, some increase in the size of the ovary, and slight curving and thickening of the pedicels. The response to pollen from a different plant of the same clone is more pronounced than to pollen from the same plant.

A study of open-pollinated umbels indicated that poor setting of fruit cannot be attributed to lack of adequate pollination, though lack of compatible pollen may be a contributing cause.

Pollen tubes from a single pollinium or stigmatic cavity all enter one style. If both carpels are to develop there must be germinated pollinia in two or more cavities and these cavities must be oriented in such a way as to feed both carpels.

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